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TITLE: COMPARISON OF REMOTELY ACQUIRED DEEP-BODY AND SUBDERMAL TEMPERATURE MEASUREMENTS FOR DETECTING FEVER IN CATTLE

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COMPARISON OF REMOTELY ACQUIRED DEEP-BODY AND SUBDERMAL TEMPERATURE MEASUREMENTS FOR DETECTING FEVER IN CATTLE

G. L. Seawright, R. R. Brown, K. Campbell, R. L. Levings, and C. T. Araki

A preprototype animal electronic identification (EID) system has been developed in our laboratory (Koelle et al. 1975 and Bobbett et al. 1977) and subjected to field testing for about 3 years (Anderson et al. 1981). The system uses an implantable, passively powered transponder that can be remotely interrogated to report temperature and identification. With commercial versions of the system, the identification feature will provide animal management and inventory benefits, and the temperature feature may provide a means for detecting disease, stress, ovulation, and parturition (Holm 1981 and Seawright 1977).

Because the transponder receives its power from an external rf source, it must be implanted superficially under the skin, rather than in deep tissues, to minimize attenuation of the powering beam. This requirement raises the question of whether subdermal temperatures accurately reflect fever or body temperature changes that signal the presence of important physiological events. To determine the usefulness of subdermal temperature measurements for these purposes, we have conducted a series of experiments where cattle temperatures have been monitored by radiotelemetry at multiple body sites. Radiotelemetry, which utilizes battery-powered transmitters, was used because it allows continuous temperature measurements to be made simultaneously from several body sites, including those that reflect deep-body temperatures. Unlike the EID system, data can be obtained from free-ranging animals regardless of their location relative to the receiving antenna.

Our objective in these studies was to determine whether fever can be detected in cattle by measuring temperatures at various subdermal sites. We describe here results of two studies in which deep-body and subdermal temperatures were compared with fevers that were experimentally induced with viruses. In the first study, test animals were held indoors where ambient temperatures were stable; in the second study, animals were held outdoors during the winter months when temperatures were highly variable. We also describe for the first time a computerized temperature telemetry system used for the studies.

MATERIALS AND METHODS

Two experiments were performed at different times and with different facilities, equipment, animals, and environmental conditions.

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Equipment

Transmitters (Experiment 1): Cattle were instrumented with small (16 x 23 x 100 mm), pulse-interval-modulated transmitters operating in the 148-150 MHz range (commercially obtained from J. Stuart Enterprises, Grass Valley, California). The transmitters were equipped with flexible probes constructed from ~10-cm segments of Microdot S-70 series, Teflon-coated, coaxial cables (Microdot, Inc., South Pasadena, California). Probe tips were fixed with 1-MΩ Fenwal thermistors (GA 61J1), dipped in a molten mixture of 20% Elvax vinyl resin (grade 260, Electrochemical Dept., Wilmington, Delaware) and 80% paraffin (Arco Wax, No. 1230, Atlantic Richfield, Philadelphia, Pennsylvania). Heat-shrink tubing was then applied over the thermistor, and the tip of the probe was dipped in Dow-Corning 282 medical-grade elastomer. The junction of the connector and cable was reinforced by a rubber cuff and a 3.0-cm segment of Dow-Corning medical-grade Silastic tubing. The tubing was fixed in place by Silastic medical adhesive (Type A).

Deep-body temperatures were measured with transmitters mounted on the ear pinna. Probes on the transmitters were inserted into the ear canal so that the tip with the thermistor was positioned near the tympanic membrane, where temperatures closely approximate those at the hypothalamic thermoregulatory center (Benzinger 1962). The technique for mounting the transmitters is described elsewhere (Seawright et al. 1979).

Subdermal temperatures were monitored with the same transmitters used for ear canal monitoring but were fitted with 12-in. thermistor probes. The transmitters were sutured to the skin in the dorsal postscapular region of the thorax, 3 in. ventral to the top line of the animal (withers). The thermistor probe was inserted into the bottom of a sterile, stainless steel sheath that was closed at one end. The closed end of the sheath was then inserted by blunt dissection into the subcutis through a small skin incision. The exposed part of the sheath was fitted with a sterile surgical rubber cuff, which was sutured to the edges of the skin incision [implant method of Dr. L. M. Holland, Los Alamos National Laboratory (unpublished)]. The technique produced an effective barrier against infectious tracts through the wound.

Each calf was also equipped with a third transmitter for monitoring rectal temperatures. The transmitter was taped to the dorsal aspect of the proximal end of the tail, and a 14-in. Elvax-encapsulated probe, made up by the method described for ear canal probes, was inserted into the rectum.

Receiving and Data Logging System (Experiment 1): The receiver was a modified 10-channel CB scanner (Regency Act-W10). It was interfaced to a Los Alamos designed and built microcomputer having an Intel 8080 microprocessor (Criscuolo, 1977). The microcomputer was used to select the channels and to control the sample rate and duration of measurements on individual animals. The microcomputer was also interfaced with a Texas Instruments (Model TI-733) keyboard terminal having tape cassettes for storing data and an acoustic coupler for transmitting data to a large computer facility (Seawright et al. 1978).

Transmitters (Experiment 2): Transmitters from two sources were used to obtain ear canal temperatures; the transmitters were a hybridized modification of the commercially obtained transmitter, which is described above. The modification involved the substitution of complementary metal oxide semiconductor (CMOS) components for original components wherever possible. As a result of these modifications, the transmitter package was significantly reduced in size. The hybrid transmitter is powered by a 2.8-V lithium battery that weighs about 3 g, offers high service capacity (140 mAh), is small (23 mm in diameter and 2.6 mm thick), and provides about 3 months of service with normal use. The battery and hybrid circuit are contained in a discoid housing measuring 36 mm in diameter and 8.6 mm in thickness. The housing is made of machined Kel-F (3M Company, St. Paul. Minnesota), and the total package weighs 11 gm. The transmitter package was mounted onto plastic ear tags (sheep button; Delta Plastics, Ltd., Palmerston North, New Zealand) that were first affixed to the ear pinna.

Thermistor probes were fabricated with 1-MΩ thermistors (Fenwal Electronics, Framingham, Massachusetts) soldered to Teflon-coated 32 AWG stainless steel wire (Nasco Biomed Systems, Inc., Houston, Texas). Melted polyethylene tubing was used to form a uniform, conformational coat around the wire assembly. Further details of the transmitter and probe design and fabrication can be found in the report by Araki et al. 1980.

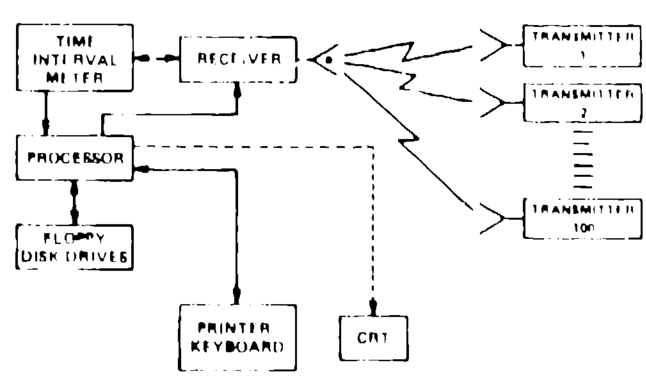
Subdermal temperatures were measured with Elvax-encapsulated transmitters (Mark IV model) commercially obtained from Telonics (Mesa, Arizona). Transmitters were surgically implanted into the subcutis in the withers region as before and in the dewlap, anterior to the brisket.

Receiving and Data Processing System (Experiment 2)

System Configuration: Each system is housed in a 19-in. rack with an overall height of 23 in. and requires a maximum of 1 kW of power. The receiving and data processing system (monitor) is a specially designed unit that receives, stores, processes, and displays telemetry data.

The major system components are shown in Fig. 1. All hardware is commercially available except the Los Alamos designed interface between the processor and the telemetry equipment. The telemetry receiving equipment consisted of a receiver and pulse-interval meter. The Transmitted signals are acquired by a Telonics TR-2 receiver (Telonics, Mesa, Arizona) which operates in the 150-to 152-MHz range. Within this range, the receiver can select 2000 discrete frequencies in 1-kHz increments. The Telonics TDP-1 pulse-interval meter accepts the data from the receiver and processes the data in a form acceptable by the computer.

Fig. 1. Block Diagram of Computerized Remote Temperature Telemetry System.



The computer has a Digital Equipment Corporation (DEC) LSI-11/2 central processing unit (CPU) with 32 kbytes of memory. Mass storage is provided by a Data Systems (Santa Clara, California) dual floppy-disk drive. The disk drive accepts 8-in. single or double-density floppy disks. The CPU uses the Teletype model 43 keyboard/printer and a DEC VT-100 video terminal.

Operating System: The operating system is responsible for communicating with the operator and implementing the commands issued by the user. When the monitor is turned on, the operator receives a short message and then a prompt. The prompt indicates that the monitor is ready for a command. A command such as an initialization request, will cause the monitor to query the operator about the data-acquisition parameters necessary for a run (scan rate, transmitter frequencies, temperature conversion coefficients, etc.). Each response by the operator is checked for format and to see that it is within prescribed limits before the monitor will accept it. All of the commands can be issued while a data-acquisition run is in progress. This enables the operator to observe the data collection results as they are occurring.

The operating system checks the transmitted data to determine if any transmission errors have occurred. The transmission error bounds are set in the initialization sequence and include upper and lower temperature values that are considered valid and the maximum change in temperature value that is likely to occur from one data point to the next. When an error is detected, the operating system notifies the user of the error condition in subsequent data print-outs. In addition, a time-referenced log is kept of all the transmission errors occurring during the data-acquisition run. This log can be observed by the operator at any time.

The operator can also place markers in the data print-outs. These markers flag events that the user decides are significant. For example, a marker may be set for a particular cow when it is vaccinated if the operator suspects the vaccination will affect the next temperature reading. The researcher is then alerted to this situation on subsequent data print-outs and can interpret the data accordingly.

The operating system for the monitor was developed at Los Alamos. To facilitate future improvements on the operating system, the program is fully commented and uses FORTRAN IV.

Scan Options: During data acquisition, frequencies are automatically scanned at a fixed rate. The scan rate can be in 1-min increments for a duration of 1 to 1440 min (24 h). During each scan, all transmitters are accessed in that sequence specified during initialization. A given transmitter is specified by the cow number and the location of the temperature probe in the cow. This information is included when the operator requests a display of the temperature data received during a data-acquisition run. To convert raw time-interval data from each transmitter into the corresponding temperature values, a coefficient query is included in the initialization sequence. The coefficients specify a quadratic equation that converts time-interval data for a particular transmitter into temperature values.

If the computer receives no data for a particular transmitter, a "search" routine is entered. The monitor assumes the transmitter has drifted from the proper frequency and therefore examines alternative frequencies for data. When the correct frequency is found, the monitor updates the old transmitter frequency with the correct one and then continues the scanning sequence.

After each scan, temperature data are stored on a floppy disk. Along with the temperature data, all parameters governing the data-acquisition run are stored on the disk so that a power failure will not destroy the results of the run. Furthermore, when ac power is restored, the monitor records the power failure and goes back into operation without operator intervention.

Data Processing: Data processing includes any mathematical operations done on the temperature data. The operator has a choice of four data-processing commands: two for data conversion and two for statistical functions. The commands invoke the following functions: (1) time-interval-to-temperature conversion, (2) temperature-to-time-interval conversion, (3) statistical mean over time period, and (4) interval mean over time period.

The statistical functions are included to help the researcher recognize trends. The calculation of a statistical mean over a given time period makes use of all data points included in the given time period. The time period is specified by the operator in terms of a start and stop time. The calculation of the interval mean makes use of a group of mean values over the time period of interest. The overall time period is divided into equally spaced intervals, and the mean value is calculated for each (daily mean values could be calculated over the course of a month, for example). For the interval mean, the operator specifies the interval time period and the overall time period. The statistical function commands can be applied to a single transmitter or to all transmitters. Any data point that is associated with a transmission error will not be included in the statistical calculations.

Data Display: There are two formats that can be used to display data: plot format and table format. A table listing will display the data from a maximum of 100 transmitters whether the data are time interval, temperature, or statistical data. All listed data are time-referenced and include any system-generated or operator-generated markers. Each page begins with a header and is followed by the columns of time and date entries. The page header includes the time and date of the listing, the page number, the cow number, and the transmitter location.

For an up-to-date account of the data-acquisition scan parameter, an information header can be listed at any time. The information header consists of the time and date of the listing, the scan rate, the cow number, the transmitter-location-to-transmitter-frequency correlation, a list of the quadratic coefficients for each transmitter, the data-acquisition run start time, and the present status of the run. Included in the run status is an estimate of the amount of time remaining until the monitor must abort the run for lack of storage space on the disk.

Plots are available in the form of data versus time. The data can be temperature data or statistical means. The operator has the choice of plotting the data from a single transmitter or from several transmitters on the same plot.

For general use by the operator, the monitor provides a "scratch pad" area within the computer. This area can be used to log comments pertinent to the data-acquisition run. The comment log can be displayed at any time by issuing the appropriate command.

Experimental Design and Analysis

Experiment 1: Experiment 1 was conducted with 5-month-old steers that were housed together in an isolation facility at the National Veterinary Services Laboratories in Ames, Iowa. Temperatures in the facility were maintained at 18 ± 2.5°C. The steers were instrumented for deep body, subdermal, and rectal temperature measurements, as described above, and remote temperature data were collected from each site at 5-min intervals.

After a 24-h lead-in time, each calf was intranasally inoculated with 1 mℓ/nostril of a suspension of bovine virus diarrhea (BVD) virus (New York strain) consisting of $10^{6.0}$ plaque-forming units (PFU) of virus made up in minimum essential medium with Hank's balanced salt solution.

Experiment 2: Experiment 2 was conducted with 2-year old steers that were held outside in a fenced enclosure near Los Alamos, New Mexico. The experiment was conducted during December and January when ambient temperatures averaged 14.2°C and 9.2°C, respectively, and ranged from -16.1°C to 16.1°C in December and from -16.1°C to 12.2°C in January. Average relative humidity in December and January was 47% and 60%, respectively. The steers were instrumented for deep-body temperature measurements and for subdermal measurements in the dorsal postscapular and dewlap regions as described above. Temperature data were collected from each site at 10-min intervals.

After a 24-h lead-in time, each steer was intranasally inoculated with 1 mℓ/nostril of a suspension of infectious bovine rhinotracheitis (IBR) virus consisting of $10^{8.0}$ PFU/mℓ of virus made up as before.

Editing of data: The data were edited to remove outlying observations and to fill in short gaps. Points outside reasonable bounds, based on inspection of the bulk of the series, were eliminated as outliers. Also eliminated were points representing too great a change since the preceding valid observation. If backup data were available (during the experiment, two systems were operated in parallel), these were used to fill in gaps. Remaining gaps, up to about 2 h, were filled in by linear interpolation.

Data analysis: Two types of analysis were performed on the edited data. First, assuming that the subdermal temperature s(t) depends on the ambient temperature at time t, a(t), and at earlier times t-h as well, we fitted a model of the form

$$s(t) = \sum_{k=0}^{n} \theta_k \, a(t - k\Delta t) + r(t) \quad , \tag{1}$$

where $\Delta(t)$ is the scan rate and the coefficients θ_k were chosen to minimize the variance of the residual series r(t). [Equation (1) implies a causal relationship between ambient temperature and subdermal temperature.] Ideally the residual series would be free of ambient temperature effects and follow more closely the animal's deep-body temperature.

The second type of analysis arose from the observation that each day's peak subdermal temperatures appeared to reflect the trend of the fever as measured from the ear canal. Details of the analysis are described below under Experiment 2.

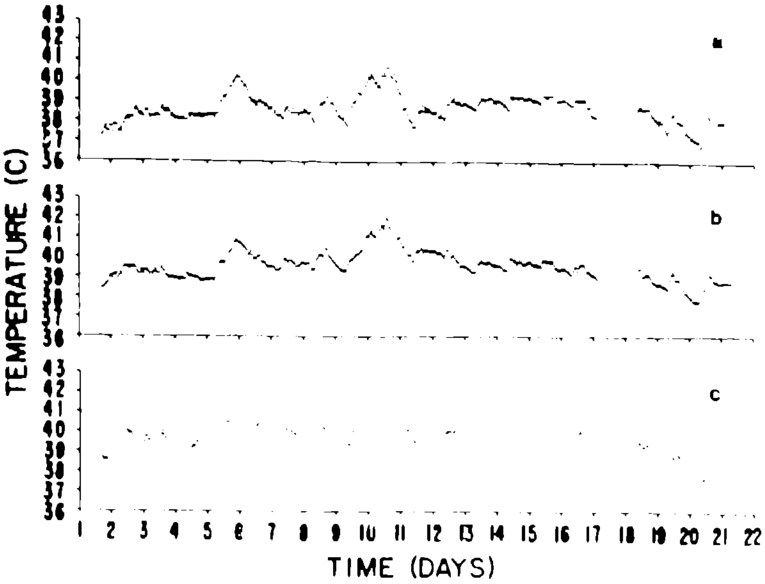
RESULTS AND DISCUSSION

Experiment 1

Figures 2 (calf 8323) and 3 (calf 44) show continuous temperature plots for tympanic membrane and subdermal temperatures and semicontinuous plots for rectal temperatures over a period of 22 days. The semicontinuous nature of the rectal temperature data resulted from the probes being voided and reinstalled several times daily. Figures 2b and 3b show tympanic membrane temperature that, because of less lag time and proximity to the hypothalamic thermosensitive sites, is a better measure of deep-body temperature than rectal temperatures (Benzinger 1962). Diurnal rhythm accounts for many of the temperature variations in these plots.

The data in Fig. 2 show that day 5 is the beginning of the first peak of a diphasic fever (calf 8323), which by day 7 had subsided to normal. From days 8 to 9 a diurnal variation of 1.5°C was observed, and this was followed on day 10 by the second peak of the diphasic fever. The first and second peaks were 3.0 and 3.5°C above normal, respectively. Figures 2a and 2b show that the times and amplitudes of subdermal and tympanic temperature variations were in close agreement, but subdermal temperatures were generally 0.5-1.0°C lower than their tympanic counterparts. Figure 2c shows that rectal temperature profiles were in fair agreement with tympanic and subdermal temperatures, but the discontinuous nature of the former makes direct comparison difficult.

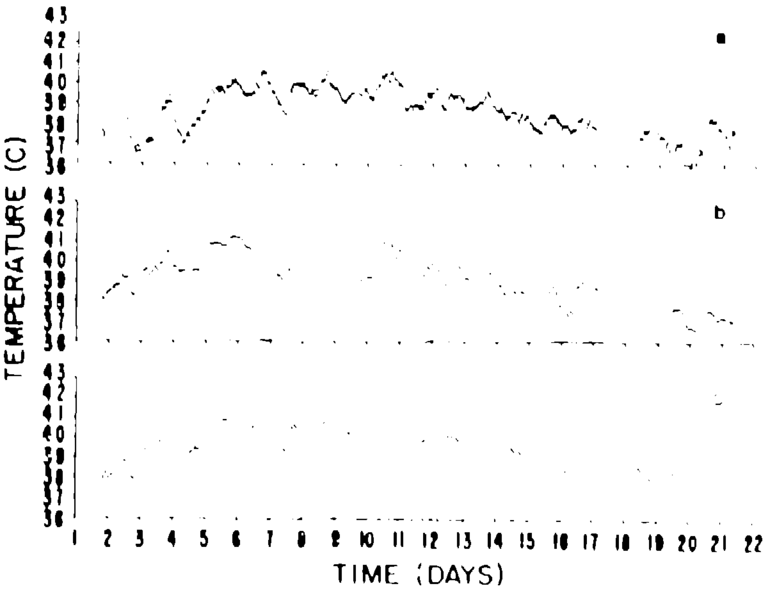
Fig. 2. Subdermal (a), Tympanic Membrane (b) and Rectal (c) Temperature Plots for Calf 8323, which Was Experimentally Infected with BVD Virus. Points are Hourly Means of 5-min Interval Measurements.



The data for calf 444 (Fig. 3) show a much broader febrile response and one with less pronounced diphasic characteristics than those seen in calf 8323 (Fig. 2). Again, the patterns for tympanic, subdermal, and rectal temperatures were generally similar. However, some interesting differences between subdermal and tympanic diurnal variations can be seen from the data in Figs. 3a and 3b. The plot of subdermal temperatures shows remarkably large (2.75°C) diurnal variations on days 3 and 4, just before the ascending limb of the first fever peak. By contrast, diurnal variations shown in the tympanic plot (Fig. 3b) are only 1°C. This difference must be viewed with caution as it was not seen in calf 8328. Further, there is not enough preinfection lead-in data to make valid comparisons with the calf's normal diurnal temperature patterns. However, if further experiments show that this event commonly occurs in cattle undergoing infectious disease processes, it may serve, at least in some animals, as an early harbinger of disease.

Another difference in tympanic and skin diurnal variations occurred from days 6 to 11, between the first and second diphasic fever peaks,

Fig. 3. Subdermal (a), Tympanic Membrane (b), and Rectal (c) Temperature Plots for Calf 444, Which was Experimentally Infected with BVD Virus. Points are Hourly means of 5-min Intervals.



respectively. In this instance the diurnal variations were greater with tympanic than with skin measurements. This may reflect some fundamental interplay between the skin and central thermoreceptors in the maintenance of the febrile state. Although conclusions cannot be made from the limited data, further investigation of this phenomenon would be in order.

The data from both calves indicate close agreement in the amplitude and temporal relationship of skin temperatures with deep-body temperatures measured near the hypothalamic thermoregulatory center and in the rectum. The data may add credence to the proposed use of subdermal temperature measurement from electronic identifiers for disease detection, at least where animals are held in a semicontrolled temperature environment.

Experiment 2: Only data from one of the two steers are shown here, but information from the second steer is similar. The data in Fig. 4 show normal body temperature patterns obtained during a 10-day runout period beginning about 12 days after the fever had abated (days 42 to 52 of the experiment). Subdermal temperatures at the withers and dewlap sites show wide swings each day; daily variations from the subdermal sites were as large as 17°C (withers) to 40°C (dewlap). By contrast, tympanic temperatures remained relatively stable throughout the observation period. Diurnal variations of about 1°C were observed; temperature minima and maxima occurred during the early morning hours and at midday, respectively.

On the assumption that the large variations in subdermal temperatures were caused by the large swings in the ambient temperatures, a filtered version of the ambient temperatures was used as a regressor to reduce the variance of the subdermal temperatures. The resulting residual series from the withers data was derived from Eq. (1). This regression reduced the variance of the total series by about one-third, but the results (Fig. 5) still have far more variability than the tympanic temperatures.

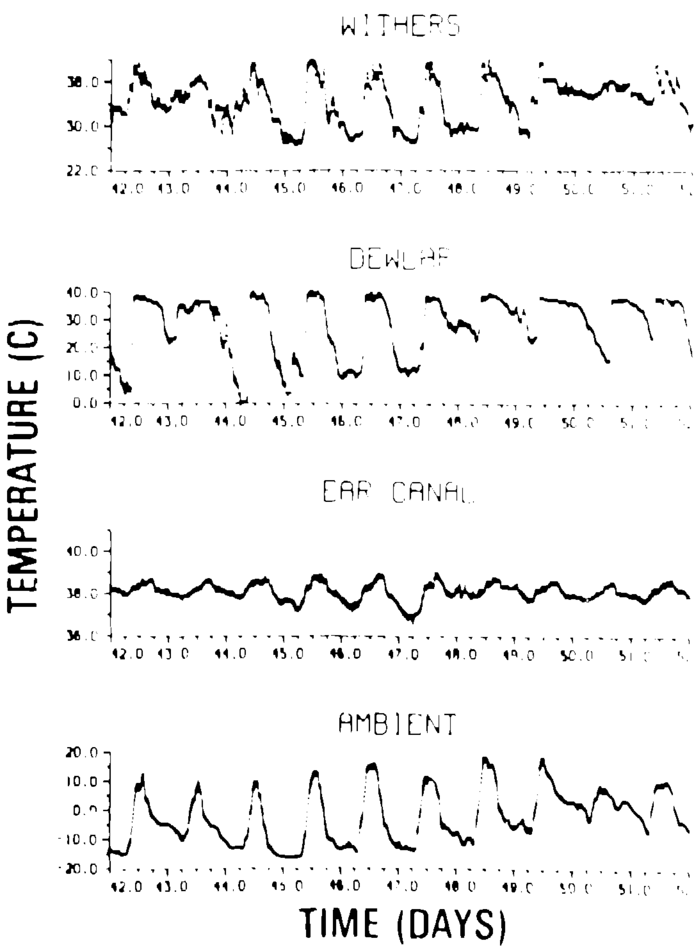


Fig. 4. Withers, Dewlap, Tympanic (Ear Canal), and Ambient Temperature Plots for the Post-Fever Period. Points are 10-min Interval Measurements

Fig. 5. Unfiltered Withers, Tympanic (Ear Canal), and Ambient Temperature Data Compared with Filtered (Residual) Withers Temperature Data for the Post-Febrile Period of a Steer Experimentally Infected with IBR Virus. Points are 10-min Interval Measurements.

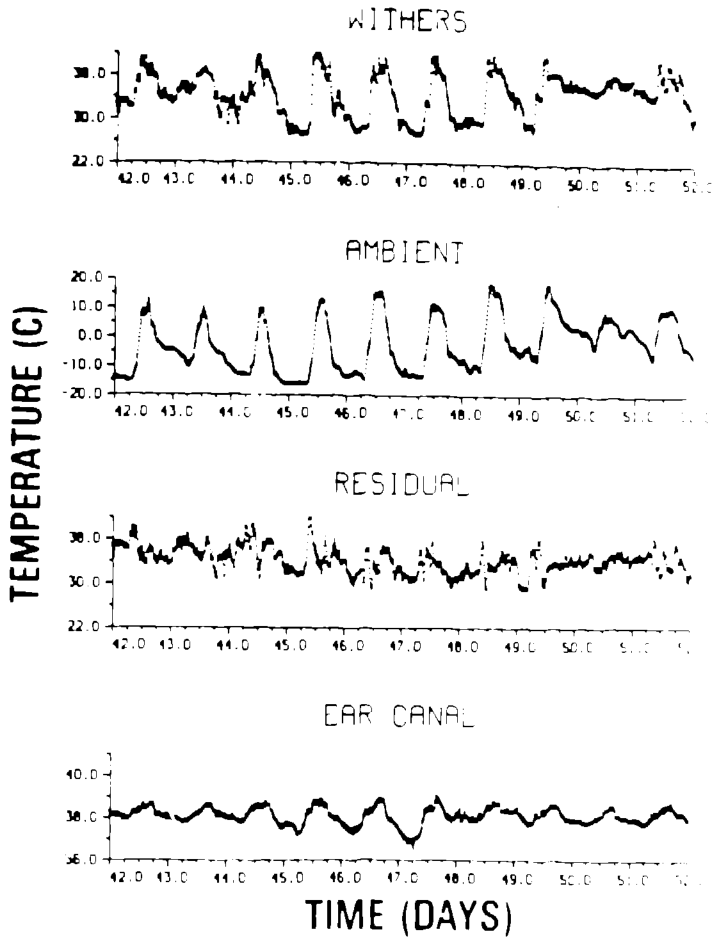


Figure 6 shows subdermal and tympanic temperature patterns during the febrile period (days 22 to 32). Temperature data from the ear canal show a clear-cut febrile response that peaked at 3°C above normal and lasted for 5 days (days 25-30). However, data from the withers did not reveal the fever either in the unfiltered series (Fig. 6) or in the residual series after (partial) removal of ambient temperature effects (Fig. 7).

The fever was also not detectable in the unfiltered dewlap data (Fig. 6), but because the relationship between the dewlap and ambient temperatures was highly nonlinear (Fig. 8), no attempt was made to fit the model of Eq. (1) to this series. Furthermore, the large swings in the dewlap temperatures (especially the morning increase) sometimes precede the corresponding changes in the ambient temperature, bringing into question the causal form of that model.

Because the above approach to data analysis did not produce meaningful results, an alternative method was attempted for detecting fever. The alternative method of analysis arose from the observation that to a limited extent, each day's peak subdermal temperatures seemed to reflect the trend of the fever as measured from the ear canal (Fig. 9). Accordingly, one-hour averages (i.e., averages over six consecutive observations) were computed at the same time each day (between 1130 and 1230 in Fig. 10). These averages seem to be uncorrelated with the corresponding ambient temperatures. The dewlap averages do a fair job of tracking ear canal temperatures, but even these averages contain enough noise to mask the effect of fever; single observations at a fixed time each day are even more variable.

From the data in Experiment 2, it would not be reasonable to expect subdermal temperatures *per se* to be a reliable method for detecting fevers or the more subtle temperature changes that accompany physiological events

Fig. 6. Withers, Dewlap, Tympanic (Ear Canal) and Ambient Temperature Plots for the Febrile Period of a Steer Experimentally Infected with IBR Virus. Points are 10-min Interval Measurements.

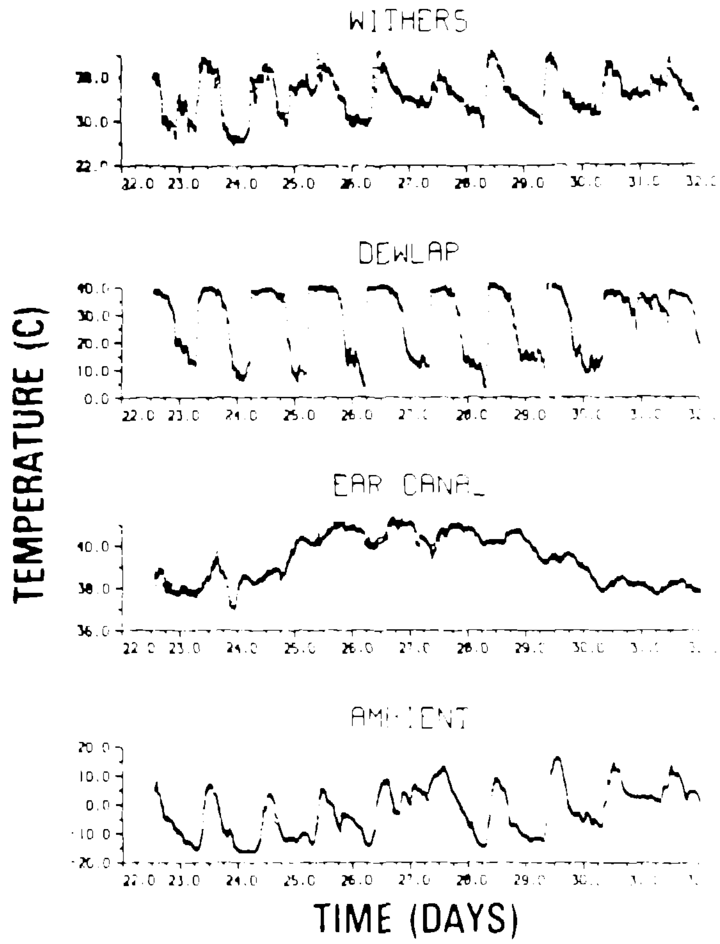


Fig. 7. Unfiltered Withers, Tympanic (Ear Canal), and Ambient Temperature Data Compared with Filtered (Residual) Withers Temperature Data for the Febrile Period of a Steer Experimentally Infected with IBR Virus. Points are 10-min Interval Measurements.

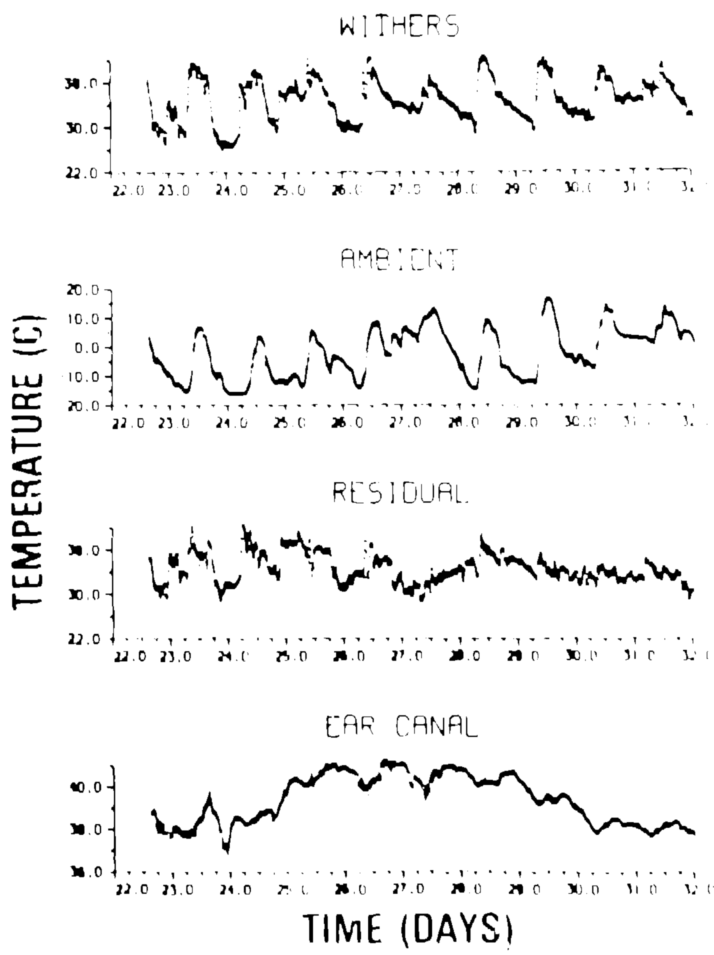
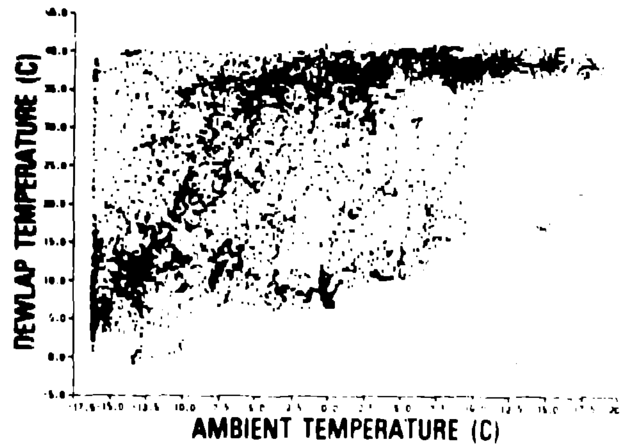


Fig. 8. Scatter Plot Showing Dewlap vs Ambient Temperature Data.



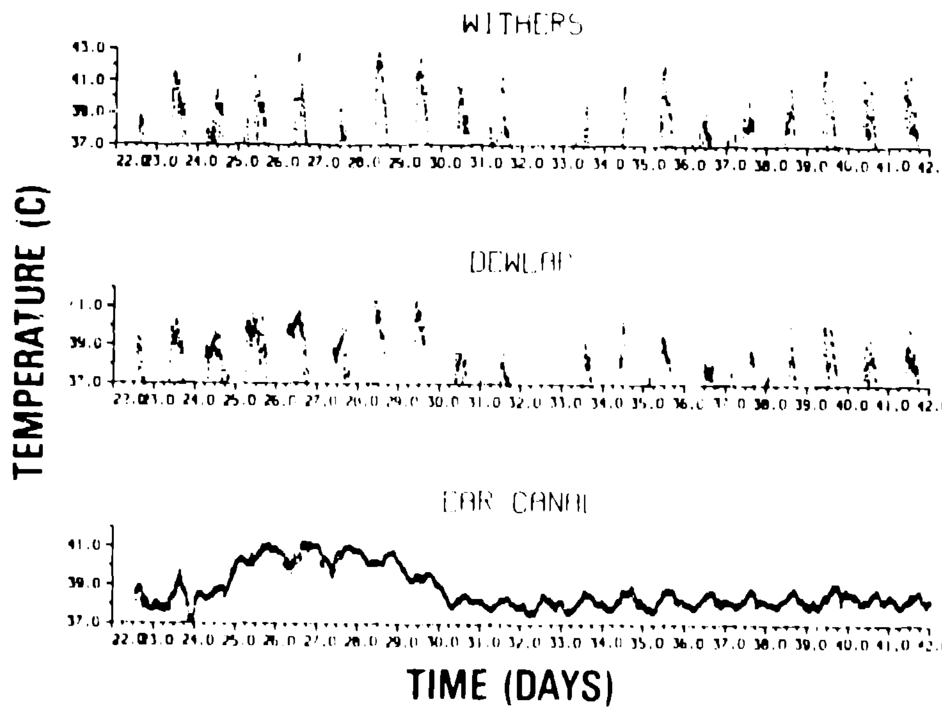
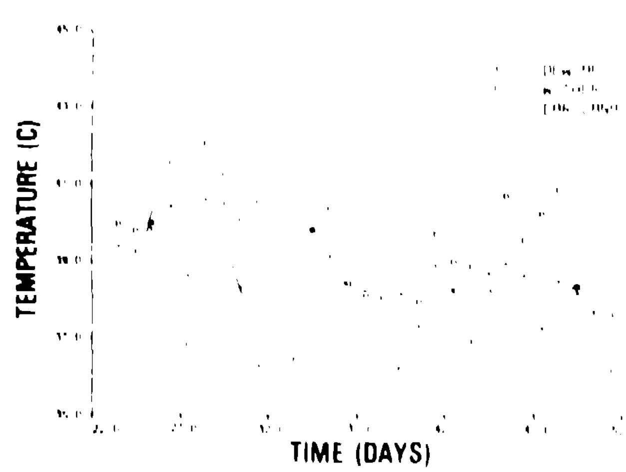


Fig. 9. Midday Temperatures for the Withers and Dewlap Subdermal Sites and for the Tympanic (Ear Canal) Site During a Period of Fever in a Steer Experimentally Infected with IBR Virus.

Fig. 10. Hourly Temperature Means Measured at the Withers, Dewlap, and Tympanic Membrane (Ear Canal) During a Period of Fever in a Steer Experimentally Infected with IBR Virus.



such as ovulation or parturition. Although subdermal and core temperature patterns were almost identical under the controlled ambient temperature conditions of Experiment 1, such conditions will not often prevail in most livestock-rearing environments.

In the past, there has been a fair amount of enthusiasm about the advantage of temperature monitoring with implantable electronic identifiers in livestock. However, with a passively powered system, which requires that implants be placed subdermally to minimize attenuation of the powering beam through the tissues, further work will be needed on antenna design and implant geometry before these advantages are realized.

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